Highlights

- HPH promotes tomato matrix disruption and consistency increase
- HPH decreases carotenoid bioaccessibility in tomato
- Tomato consistency is inversely correlated with carotenoid bioaccessibility
- Bioaccessibility depends on both tomato variety and carotenoid species

Microstructure and bioaccessibility of different carotenoid species as affected by high pressure homogenisation: a case study on differently coloured tomatoes

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Abstract

The effect of high pressure homogenisation (HPH) on structure (Bostwick consistency, particle size distribution and microstructure) and carotenoid *in vitro* bioaccessibility of different tomato pulps was investigated. HPH decreased tomato particle size due to matrix disruption and increased product consistency, probably due to the formation of a fibre network. Homogenisation also resulted in a decrease of *in vitro* bioaccessibility of lycopene, ζ-carotene, and lutein. Such decrease was attributed to the structuring effect of HPH. An inverse relation between tomato consistency and carotenoid in vitro bioaccessibility was found. This dependency was affected by carotenoid *species* and its localisation within the matrix. It could be observed that one matrix (e.g. (homogenised) red tomato pulp) can contain carotenoids with a very low bioaccessibility (lycopene) as well as carotenoids with a very high bioaccessibility (lutein), indicating that carotenoid bioaccessibility is not solely dependent on the matrix.

Keywords: high pressure homogenisation, tomato pulp, lycopene, ζ-carotene, lutein, *in vitro* bioaccessibility.

1. Introduction

High pressure homogenisation (HPH) is commonly used to increase physical stability of pumpable tomato derived products (Thakur, Singh, & Handa, 1995; den Ouden, & van Vliet, 2002). During homogenisation, tomato pulp is forced through a narrow gap undergoing high turbulence, shear, cavitation and impact. As a consequence, tomato particle structure is modified by disaggregation of cell clusters and disruption of cells (Stang, Schuchmann, & Schubert, 2001). Homogenisation has also been shown to affect the particle interactions. For the specific case of tomato, this may possibly lead to the formation of a fibre network accounting for an increase in viscosity (Bayod *et al*., 2007; Beresovsky *et al*., 1995; Lopez-Sanchez *et al*., 2011a; 2011b). It has been suggested that such structural modifications may affect the release and bioaccessibility of carotenoids from the food matrix (van het Hof *et al*., 2000).

Carotenoids are naturally occurring fat-soluble pigments responsible for tomato colour. They are well known to have beneficial effects on human health by reducing the risk of chronic diseases, including cardiovascular and gastrointestinal diseases, obesity, diabetes and certain types of cancer (Rao & Rao, 2007). Health protective effects of carotenoids strongly depend on their bioavailability and bioaccessibility. The latter is defined as the fraction of the ingested carotenoids that is released from the food matrix, incorporated into mixed micelles and thus available for intestinal absorption (Parada & Aguilera, 2007).

Among the several factors affecting carotenoid bioaccessibility, the structure of the food matrix, as well as the *species* of carotenoid, and their localisation within the plant tissue, seem to play a major role (Castenmiller & West, 1998). The reduction in particle size due to plant tissue disruption has been shown to enhance carotenoid bioaccessibility (van het Hof, de Boer, *et al*., 2000; Parada & Aguilera, 2007; Svelander, Lopez-Sanchez, Pudney, Schumm, & Alminger, 2011; Knockaert, Lemmens, Van Buggenhout, Hendrickx, &Van Loey, 2012). On the other hand, the increase in consistency, attributed to the formation of a fibre network by polymer-polymer interaction, has been demonstrated to decrease carotenoid bioaccessibility (McClements, Decker, & Park, 2009). With regard to carotenoid species, the extent of incorporation into micelles is considered to be inversely related to their hydrophobicity. For example, lutein and other xanthophylls are known to be better absorbable into micelles and thus to have higher bioaccessibility than *β*-carotene or lycopene, which are more hydrophobic (Borel, Grolier, *et al*., 1996; Schweiggert, Mezger, Schimpf, Steingass, & Carle, 2012). In plant tissues, carotenoids are generally deposited in various types of chromoplasts in which they occur in crystalline or lipid-dissolved form (Rosso, 1968; Vasquez-Caicedo *et al*., 2006). In general, the latter are easily incorporated into micelles and thus more accessible than those stored in crystalline form (Schweiggert *et al*., 2011b; 2012; Vasquez-Caicedo, Heller, Neidhart, & Carle, 2006).

In recent years, several studies have focussed on the effect of HPH on carotenoid bioaccessibility in tomato pulps as well as in other matrices. Upon HPH, a decrease in lycopene bioaccessibility was detected in both tomato pulps and tomato based emulsions. Such decrease was attributed to the formation of a fibre network (Colle, Van Buggenhout, Van Loey, & Hendrickx, 2010; Svelander *et al*., 2011). On the other hand, *β*-carotene bioaccessibility was found to increase in both carrot purees and carrot based emulsions following HPH. In these cases, the improvement in *β*-carotene bioaccessibility was associated to the disruption of the matrix (Svelander *et al*., 2011; Knockaert et al., 2012). Given these apparently contradictory evidences, it is largely unclear whether carotenoid bioaccessibility depend either on the carotenoid species or the structure of the matrix

The aim of the present study was therefore to investigate the effect of HPH on different carotenoid *species* within a similar matrix. Therefore, tomatoes with different colour (i.e. red-orange- and yellow-fleshed), and thus different prevalent carotenoids, were chosen. Samples were homogenised at increasing pressure levels and analysed for carotenoid bioaccessibility and concentration. To understand the relations between changes in carotenoid bioaccessibility and plant tissue structure upon HPH, samples were also submitted to evaluation of Bostwick consistency, particle size distribution and microstructure.

2. Materials and methods

2.1. Materials

Red, orange and yellow ripe tomatoes (*Solanum lycopersicum* L.) of the cultivars Admiro, Bolzano and Lorenzo respectively, were purchased at a local greengrocer. They were washed, wiped, manually quartered with a sharp knife, frozen in liquid nitrogen and stored at −40 °C until use. The same batch of each tomato was used for the preparation of all samples. Extra virgin olive oil was purchased in a local supermarket and stored under dark. Lycopene, *ζ*-carotene and lutein standards were purchased from CaroteNature (Lupsingen, Switserland). *β*-Apo-8′-carotenal, was obtained from Sigma-Aldrich (Bornem, Belgium).

2.2. High pressure homogenisation

The frozen tomato quarters were thawed, peeled, mixed (3 times for 5 s) (Büchi Mixer B-400, Flawil, Switzerland) and sieved (pore size 1.0 mm) in order to remove the seeds. Tomato pulps were homogenised *via* a single pass at 20, 50 and 100 MPa using a high pressure homogeniser (Panda 2K; Gea Niro Soavi, Mechelen, Belgium) with inlet and outlet connected

to a heat exchanger at a pre-set temperature of 4 °C. Non homogenised pulps were considered as control samples. All the samples were kept in the dark at 4°C until analysis.

2.3. Bostwick consistency

The consistency of tomato pulps was measured using a Bostwick consistometer. This empirical test was conducted allowing the sample to flow under its own weight along a sloped stainless steel tray for 30 s at room temperature (23 °C). The distance the pulps flowed was recorded as the Bostwick consistency index (cm). High values correspond to a low consistency pulp with low resistance to flow, while lower values are associated with high consistency pulps resistant to flow. Measurements were done in triplicate.

2.4. Particle size distribution (PSD)

The PSD of tomato pulps was measured by laser diffraction using a Malvern Mastersizer S long bench instrument (Malvern Instrument Ltd., Worcestershire, UK). Tomato pulp was poured into a stirred tank filled with water until a laser obscuration of 20% was achieved. The diluted sample was pumped into the measuring cell, which was located in the optical path of the laser beam. The laser beam (He–Ne laser, wavelength 633 nm), collimated at 18 mm, was scattered to detector units (42 element composite solid state detector array), which detected particles in the range of 0.05 to 880 μm. Volumetric PSDs were calculated from the intensity distribution of the scattered light using the Mie theory by use of the instrument software.

2.5. Light microscopy

Micrographs of tomato pulps were taken using a digital camera mounted on a light microscope (Olympus BX-41, Optical Co. Ltd., Tokyo, Japan). To visualize the microstructure of the tomato pulps, the samples were diluted 1:10 (v/v) with 0.1 % toluidine blue aqueous solution. The presence of starch was also evaluated by diluting 1:4 (v/v) tomato pulp with a iodine staining solution (0.2% iodine, 2% potassium iodide aqueous solution). Few droplets of the mixtures were placed on microscopic slides, covered with cover glasses and studied using a 10x magnification. To visualize the tomato chromoplasts, one droplet of tomato pulp was placed on a microscopic slide, covered with a cover glass and analysed using a 100x magnification.

2.6. In vitro *bioaccessibility*

The lycopene, *ζ*-carotene and lutein *in vitro* bioaccessibility was measured immediately after processing the tomato pulp by simulating human digestion in the stomach and small intestine *in vitro*. The procedure described by Moelants *et al.* (2012), based on Hedrén, Diaz, & Svanberg (2002), was followed. In particular, 5 g tomato pulp was weighted into a 50 mL capacity brown falcon tube. The sample was diluted with 5 mL NaCl/ ascorbic acid solution (0.9% NaCl, 1% ascorbic acid in water), 5 mL stomach electrolyte solution (0.30% NaCl, 0.11% KCl, 0.15% $CaCl_2 \cdot 2H_2O$, 0.05% $KHPO_4$, 0.07% $MgCl_2 \cdot 6H_2O$ in water) and 10 mL of freshly prepared oil emulsion. The latter was obtained by suspending 1% (w/v) L-α-phosphatidylcholine from egg yolk (Sigma) in water. 5% (v/v) extra virgin olive oil was then added and the mixture was homogenized (UltraTurrax® T25, IKA® - Werke GMBH & CO.KG, Staufen, Germany) at 9500 rpm during 10 min. A second homogenization was performed at 100 MPa for one cycle using the high pressure homogeniser described above.

To simulate the first phase of gastric digestion, the pH of the mixture was adjusted to 4±0.05 with 1 M HCl or 1M $NaHCO_3$ and 5 mL pepsin solution (0.52% porcine pepsin, from Sigma, in electrolyte solution) was added. After flushing the headspace of the samples with nitrogen for 20 s, the mixture was incubated at 37 °C for 30 min while shaking end-over-end. The pH of the mixture was then acidified to 2±0.05 to mimic the drop of the gastric pH after the intake of a meal (Tyssandier *et al.*, 2003). The headspace of the samples was flushed again with nitrogen for 20 s and the incubation at 37 °C continued for another 30 min. To imitate

the passage through the small intestine, the pH of the partially digested tomato pulp was raised to 6.9±0.05 and 3 mL pancreatin/bile salts solution (0.4% porcine pancreatin, 2.5% bile extract, 0.5% pyrogallol, and 1% *α*-tocopherol, from Sigma, in water) was added. Finally, the headspace of the sample was flushed with nitrogen for 20 s and incubated for 2 h at 37 °C. The digest was centrifuged (L7 Ultracentrifuge, Beckman, Palo Alto, CA, USA) at 165,000 g during 65 min at 4 °C to separate the micelles. The supernatant was collected, filtered (Chromafil PET filters, 0.20 μm pore size-25 mm diameter) and analyzed for carotenoid content (C_m). The *in vitro* bioaccessibility of the carotenoids was defined as the percentage ratio between the carotenoid content in the micelles (C_m) and in the digest of the control sample (i.e. non homogenised tomato pulp) (C_d). The latter sample was obtained immediately after digestion (no ultracentrifugation).

2.7. Carotenoid content

The extraction of lycopene, *ζ*-carotene and lutein was performed following the procedure of Sadler, Davis, & Dezman (1990), with minor modifications. The analysis was performed under subdued light to prevent carotenoid degradation and isomerisation. 0.5 g NaCl and 50 mL extraction solution (50% hexane, 25% acetone, 25% ethanol, 0.1% butylated hydroxytoluene) were added to 2 g freshly prepared tomato pulp, to the digest of non homogenised tomato pulp or to the micellar fraction. The mixture was stirred at 4 °C for 20 min. Reagent grade water (15 mL) was added and stirring was continued for 10 min. The apolar phase was then separated from the aqueous phase using a separation funnel. The apolar phase, containing the carotenoids, was collected, filtered (Chromafil PET filters, 0.20 μm pore size-25 mm diameter) and transferred to an amber HPLC vial. When necessary, the apolar phase was concentrated under vacuum at 30 °C for 35 min using a rotary evaporator. The concentrated carotenoid extracts were redissolved in hexane:dichloromethane (4:1 v/v) and transferred to an amber HPLC vial. The concentration factor was calculated by adding a known amount of *β*-apo-8′-carotenal prior to the evaporation of the apolar solvent.

The HPLC analyses were performed on an Agilent 1200 series system equipped with a diode array detector (Agilent Technolgies 1200 Series, Diegem, Belgium), according to Colle *et al.* (2010) with some modifications. Carotenoids were separated at 25 °C on a reversed phase C_{30} column (3 μm×150 mm×4.6 mm, YMC Europe, Dinslaken, Germany) coupled to its guard column. A mobile phase of reagent grade water (A), methanol (B) and methyl-t-butyl-ether (C) was used. The gradient elution was as follows: 0 min: 4% (A), 96% (B); 6-9 min: 4% (A), 81% (B), 15% (C); 21-24 min: 4% (A), 41% (B), 55% (C); 32 min: 4% (A), 31% (B), 65% (C); 34-38 min: 4% (A), 26% (B), 70% (C); 38-48 min: 4% (A), 96% (B). Lycopene, *ζ*-carotene and lutein were identified based on retention times and spectral characteristics compared to the standards (data not shown). To quantify the carotenoids, HPLC-DAD peak responses were measured at 472 nm for lycopene, and at 450 nm for *ζ*-carotene and lutein. The carotenoid content was calculated based on their calibration curve and expressed as μg/g tomato pulp.

2.8. Data analysis

Results obtained are expressed as mean of three replicates ± standard deviation. Means were compared using one-way analysis of variance (ANOVA) followed by Tukey test (Statistica 6.0, StatSoft inc., 2001). Least squares linear regression analyses were performed by using Statistica for Windows (ver. 4.5, 1993, Stat Soft Inc., Tulsa, USA).

3. Results and discussion

3.1. Effect of HPH on tomato structure

Pulps from tomatoes having different flesh colour (red, orange and yellow) were submitted to increasing homogenisation pressure and analyzed for structural characteristics. The consistency index of tomato pulps was determined using a Bostwick consistometer (Fig. 1).

Non homogenised tomato pulps of different colours presented different consistency indices, with yellow tomato being the most consistent, followed by the orange and the red tomatoes. Such differences might be attributed to the amount of starch present in the different tomato pulps. The inset of Fig. 1 shows micrographs of tomato pulps stained with iodine. The black spots represent the starch granules. Iodine staining of tomato pulps thus revealed a higher amount of starch for yellow tomatoes compared to orange and red tomatoes.

During HPH (Fig. 1), increased pressure levels resulted in lower consistency indices and thus in higher consistency values for all the tomato pulps. As reported by other authors (Colle *et al*., 2010; Svelander *et al*., 2011), the increase in consistency can be due to the formation and strengthening of a fibre network in tomato pulps under high pressure homogenisation. Despite the differences observed in non homogenised samples, the trend of the consistency increase was similar for all the tomato varieties.

As HPH is known to induce mechanical disruption of the food matrix, tomato pulps were analysed for their particle size distributions (PSDs) (Fig. 2). In the non homogenised red tomato pulp, particles with mean diameter around 600-700 μm were found. Nevertheless, the non homogenised orange and yellow samples presented particles with smaller mean diameter (300-500 μm). As expected, the increase of homogenisation pressure induced a progressive decrease in particle dimensions. After homogenisation at 100 MPa the mean particle diameter in red tomato pulps was about 100-200 μm. Moreover, the higher the homogenisation pressure the narrower the PSD and thus the more uniform the tomato particles. The same behaviour was observed in the orange and yellow tomatoes. However, in these cases the decrease in particle dimension with homogenisation pressure was less gradual than in red tomato.

PSD results are consistent with the observations made under light microscope (supplementary material). In the non homogenised pulp of red tomato, cell clusters consisting of several cells were observed. Their presence accounts for the higher mean particle diameter (Fig. 2). Upon homogenisation at 20 MPa, only single cells and broken cell material were observed in the sample. Homogenisation at 50 MPa led to further disruption of cell material and only cell fragments were observed in the samples. At 100 MPa the complete breakage of cells occurred and cell material was uniformly distributed. In the non homogenised orange and yellow tomato pulps, only single cells and fragments of cell material were observed while no clear clusters were visible. The complete cell disruption and the release of the cellular content occurred by homogenisation at only 20 MPa and no clear additional structure modifications were observed on further increase in homogenisation pressure.

3.2. Carotenoid content and localisation in tomatoes

In order to determine the type and quantity of carotenoids present in red, orange and yellow tomatoes, HPLC analyses were performed on the corresponding non homogenised pulps. Results are shown in table 1. As expected, chromatograms showed the presence of lycopene (λ_{max}: 444; 471; 502 nm; retention time: 36 min) and lutein (λ_{max}: 421; 444; 472 nm; retention time: 10 min) in red and yellow tomato respectively. *β*-Carotene was detected as well in the red tomatoes, but due to its low concentrations compared to lycopene, no further attention was payed to *β*-carotene in this study. The chromatograms relevant to orange tomato showed the presence of one peak with retention time similar to that of *β*-carotene (retention time: 23 min) but a completely different spectrum (λ_{max}: 379; 400; 425; 462 nm). By comparison with literature information (Mackinney & Jenkins, 1949; Tomes, Quackenbush, Nelson, & North, 1953) and spectral characteristics (comparison with standards, data not shown), this peak was tentatively attributed to the elution of *ζ*-carotene (λ_{max}: 379; 400; 425 nm). Since the spectral characteristics revealed the presence of a second component, it is likely that, together with *ζ*-carotene, other carotenoid compounds (such as prolycopene) are eluted with the same retention time (Tomes *et al*., 1953).

Since digestive enzymes could promote the release of carotenoids from the matrix, analyses of the carotenoid content were also performed after digestion of the different tomato pulps (Table 1). No differences for lycopene and ζ-carotene content in the pulp and in the digest were observed. Lycopene and ζ-carotene, being carotenes, do not contain oxygen groups which can form esters. The latter could hinder carotenoid extraction from the food matrix, giving reason for the difference in carotenoid amount between the digest and the pulp. As regards lutein in yellow tomatoes, the content in the digest was found to be two times higher than in the pulp. In red and orange tomato, lutein was detected only in the digest. It was speculated that lutein might be embedded in the matrix (xanthophylls can form esters more easily due to the presence of oxygen groups) and is thus hardly extractable by hexane solely. Comparing the different tomato varieties, lutein content in red and yellow tomatoes was similar and twice as much as the amount that was observed in the orange tomato digest.

Light microscopy was also performed to visualise the carotenoid containing chromoplasts in the different tomato varieties (Fig. 3). Two types of chromoplast structures were observed in the red tomato pulp: red, large and needle-shaped chromoplasts and yellowish round-shaped chromoplasts. The former type was largely the most abundant. Based on the colour and shape as well as by comparison with literature evidences (Jeffery, Holzenburg, & King, 2012; Schweiggert et al., 2011a; Schweiggert et al., 2011b), it was speculated that the needle-shaped structures contain crystalline lycopene, and the yellowish round-shaped structures contain lipid dissolved lutein. However, further analysis could be performed to confirm this hypothesis. Upon homogenisation, chromoplasts containing crystalline lycopene were reduced in size, while no differences were observed for the globular lutein containing ones. It can be hypothesised that crystalloid chromoplasts are more fragile than the globular ones and thus more prone to mechanical rupture. In orange and yellow tomato, only round-shaped chromoplasts probably containing lipid dissolved ζ-carotene and lutein respectively were present. In the homogenised samples of both varieties, chromoplasts were found to be distributed all over the medium as a consequence of matrix disruption, but no changes in their dimension were observed.

3.3. Effect of HPH on carotenoid bioaccessibility

The effect of HPH on the bioaccessibility of the carotenoids occurring in the different tomato varieties was studied (Table 2). Interestingly, as regards the non homogenised tomato pulps, the relative amount of lycopene and ζ-carotene incorporated into micelles was about 13 and 30% respectively. By contrast, the average content of lutein in micelles was *circa* 88% for the different tomatoes. These differences might be explained by considering the nature of the carotenoid compounds and the structure of the chromoplasts in which they are embedded in the different tomato varieties (Fig. 3). Among the carotenoids under consideration, lycopene is, indeed, the most hydrophobic followed by ζ-carotene and lutein. The higher incorporation of lutein into mixed micelles compared to ζ-carotene and lycopene is in agreement with previous studies demonstrating that the extent of incorporation of lipophilic compounds into mixed micelles was inversely related to their hydrophobicity (Borel *et al*., 1996; Schweiggert, Mezger, Shimpf, Steingass, & Carle, 2011). Therefore, despite its larger total amount (Table 1), lycopene turned out to be the carotenoid, which is the least bioaccessible. The molecular structure of ζ-carotene is similar to that of lycopene. Nevertheless, microscopy analysis highlighted a marked structural difference between lycopene and ζ-carotene bearing chromoplasts, the former being crystalloid and the latter being globular. Literature data indicate that the higher incorporation into mixed micelles of ζ-carotene can be explained by the better release of carotenoids from non-crystalline chromoplasts (Vásquez-Caicedo *et al*., 2006; Schweiggert, Steingass, Heller, Esquivel, & Carle, 2011). The present results show that HPH resulted in an overall decrease of carotenoid *in vitro* bioaccessibility for all carotenoids and for all matrices. Lycopene and ζ-carotene bioaccessibility in red and orange tomatoes respectively decreased as homogenisation pressure increased up to 50 MPa. No significant

differences were observed on further increasing homogenisation pressure. Similarly, lutein bioaccessibility was found to decrease in red and yellow tomatoes upon HPH at increasing pressure levels. A slight decrease could also be observed in orange tomato, however the differences between the non- and the homogenised samples were not significant. The low amount of lutein measured in orange tomato (Table 1) could impair bioaccessibility data accuracy, giving reason for the apparent limited effect of HPH.

Based on the present HPLC data and in agreement with literature results (Colle *et al*., 2010; Svelander *et al*., 2011), the decrease in carotenoid bioaccessibility upon HPH (Table 2) can not be attributed to their loss or isomerisation. By contrast, it is more reasonable to hypothesise a relation between structural modifications and carotenoid *in vitro* bioaccessibility (Parada & Aguilera, 2007).

3.4. Relation between structural changes and carotenoid in vitro *bioaccessibility*

The data obtained demonstrate that HPH affects the tomato matrix by means of two counterbalancing effects: (i) the disruption of the matrix (Fig. 2 and supplementary material) which facilitates carotenoid release; (ii) the increase in consistency (Fig. 1) due to the formation and strengthening of a fibre network entrapping carotenoids (Colle *et al*., 2010; McClements *et al*., 2009). The observed decrease in carotenoid *in vitro* bioaccessibility with increasing homogenisation pressure suggests the structure enabling effect of HPH to dominate on the disruption one. In order to clearly observe this relationship, The relative *in vitro* bioaccessibility of lycopene, *ζ*-carotene, and lutein was thus plotted against the average Bostwick consistency of tomato pulps (Fig. 4). Regression lines, equations and determination coefficients values (R^2) are also shown. The high values of the determination coefficients (R^2) confirm the relation between the amount of carotenoids incorporated into micelles and the Bostwick consistency index. Fig. 4 clearly shows that the values of the slope (*β*) of all the regression lines are positive, indicating this relation to be positive. Basically, the higher the consistency of the matrix the lower the bioaccessibility of carotenoids. Analogous results were also reported by Colle *et al*. (2010) and Anese *et al*. (2013). These authors observed a decrease in lycopene *in vitro* bioaccessibility upon process induced structurisation of tomato pulps.

The dependence of bioaccessibility on Bostwick consistency (Fig. 4) was also affected by both the tomato nature and the carotenoid species. As regards red tomato, the increase in consistency upon HPH had minor effects on lycopene bioaccessibility (*β*=1.02) while it considerably affected that of lutein (*β*=3.11). Although HPH seemed not to modify the structure of lutein bearing chromoplasts (Fig. 3), the possible formation of a fibre network could impair lutein release from the matrix. By contrast, HPH would lead to the breakage of crystalloid lycopene chromoplasts (Fig. 3), possibly lowering the effect of the network formation on its bioaccessibility. In orange tomato, a similar dependence of *ζ*-carotene and lutein bioaccessibility on the Bostwick consistency was observed (*β*=1.91 and 1.83 respectively), probably due to the similar structure of the chromoplasts they are embedded in. The highest dependence of bioaccessibility on the Bostwick consistency was found for the lutein in the yellow tomato. In this case, the fibre network formed upon HPH seemed to markedly hinder the release of lutein from the matrix. Nevertheless lutein *in vitro* bioaccessibility was found to be always higher than *circa* 70%.

A remarkable observation in this study is that in one matrix, i.e. red (homogenised) tomato, both carotenoids with a very high bioaccessibility (lutein) and carotenoids with a very low bioaccessibility (lycopene) are present. This suggests the carotenoid bioaccessibility to be not dependent on the matrix solely. The importance of the carotenoid species for the bioaccessibility can clearly be seen in Fig. 4, since it shows that different types of carotenoids were clearly separated into two groups: lycopene and *ζ*-carotene in the lower part (low bioaccessibility), and lutein in the upper part of the graph (high bioaccessibility). In general it

can thus be concluded that the matrix effects, as well as carotenoid characteristics are important factors for carotenoid bioaccessibility.

Conclusions

Present results demonstrate that HPH of tomato pulps results in a decrease in the relative bioaccessibility of all the carotenoids. Such decrease was attributed to the formation and strengthening of a fibre network dominating on the effect of matrix disruption. Nevertheless, the differences among the carotenoid species and tomato varieties suggest that considering the structure enabling effect solely is not sufficient to predict the consequences of the process on carotenoid bioaccessibility. A particular matrix can contain different carotenoid species, which can have considerably different bioaccessibility. This was e.g. the case for the red tomatoes. On the other hand, different tomato varieties can have very different structural characteristics. Therefore the characterisation of other factors, such as the activity of endogenous enzymes, as well as the composition of the cell wall and membranes, would deliver useful additional insights into the effects of HPH on carotenoid bioaccessibility. The design of a process aimed to improve both the structural and nutritional properties of plant-based foods should therefore take into account the complex interplay of these factors.

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Table 1. Content of the main carotenoids in pulp (C_0) and digest (C_d) of non-homogenised red, orange and yellow tomatoes.

		C_0 (µg/g pulp)	C_d (µg/g pulp)
Red tomato	Lycopene	45.77 ± 2.41 [a]	44.57 ± 3.02 [a]
	Lutein	n.d.	1.48 ± 0.06
Orange tomato	ζ-carotene	6.62 ± 0.72 [a]	7.43 ± 0.81 [a]
	Lutein	n.d.	0.80 ± 0.10
Yellow tomato	Lutein	0.68 ± 0.13 [a]	1.70 ± 0.13 [b]

n.d. not detected

[a,b] means with the same letter in the same row are not significantly different (P<0.05)

Table 2. Relative amount of carotenoid incorporated in micelles (% C_m/C_d) of red, orange and yellow tomato pulp submitted to different homogenization pressure levels.

Tomato variety	Carotenoid	Homogenization pressure (MPa) 0	20	50	100
Red	Lycopene	13.1 ± 0.9 [a]	9.4 ± 0.7 [b]	6.7 ± 1.1 [c]	7.1 ± 0.8 [c]
	Lutein	93.0 ± 4.0 [a]	92.4 ± 5.7 [a]	78.3 ± 3.9 [b]	72.1 ± 6.7 [b]
Orange	ζ-carotene	29.4 ± 3.2 [a]	17.7 ± 1.9 [b]	14.7 ± 1.7 [c]	13.7 ± 1.6 [c]
	Lutein	74.2 ± 22.3 [a]	64.5 ± 9.0 [a]	68.5 ± 11.3 [a]	63.0 ± 13.2 [a]
Yellow	Lutein	96.2 ± 9.8 [a]	81.1 ± 8.5 [b]	69.2 ± 6.6 [b]	73.3 ± 6.0 [b]

[a,b,c] means with the same letter in the same row are not significantly different (P<0.05)

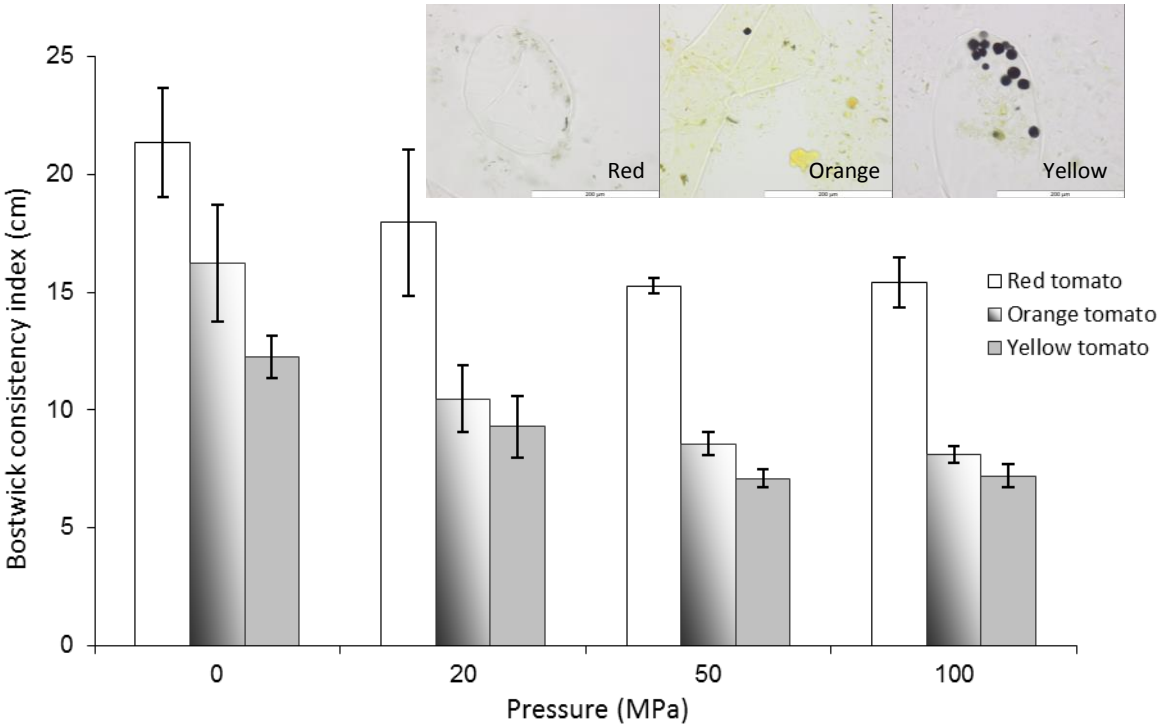


Fig. 1. Bostwick consistency indices of tomato pulp homogenised at different pressure levels. Inset: Light micrographs of non-homogenised tomato pulps stained with iodine, magnification 40x. (Arrows indicate starch granules).

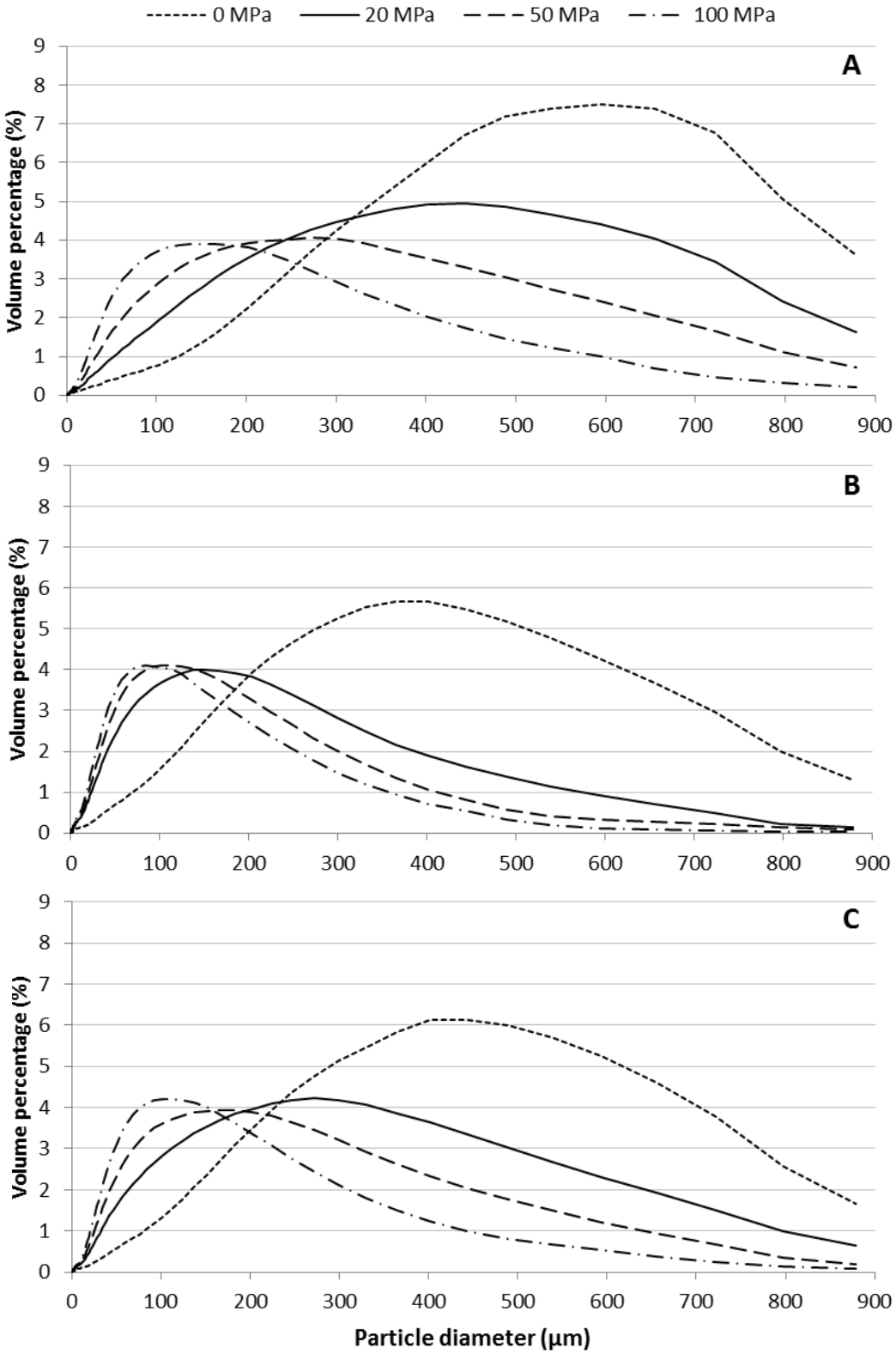


Fig. 2 Volumetric particle size distributions of red (A), orange (B) and yellow (C) tomato pulps homogenised at different pressure levels.

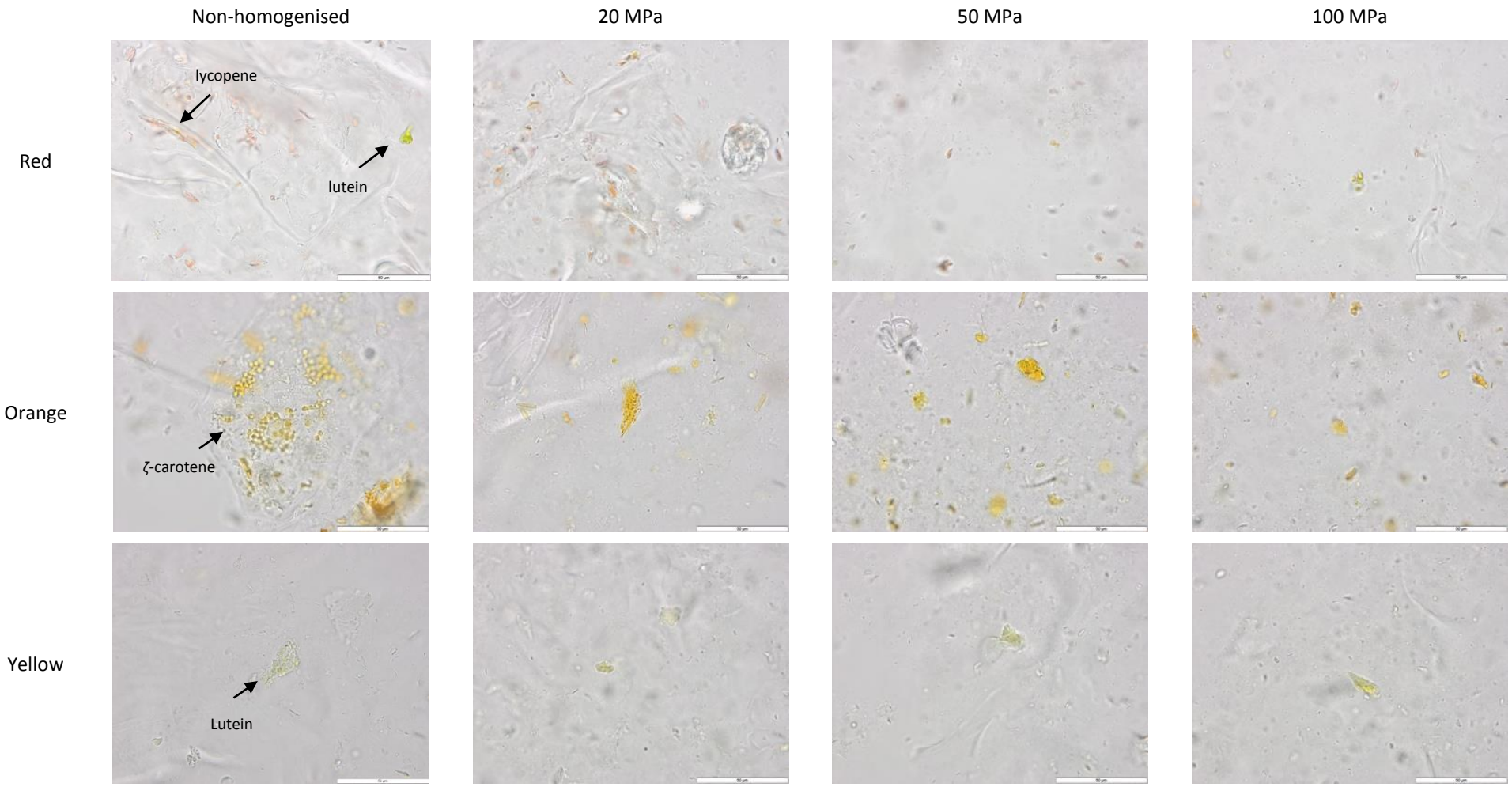
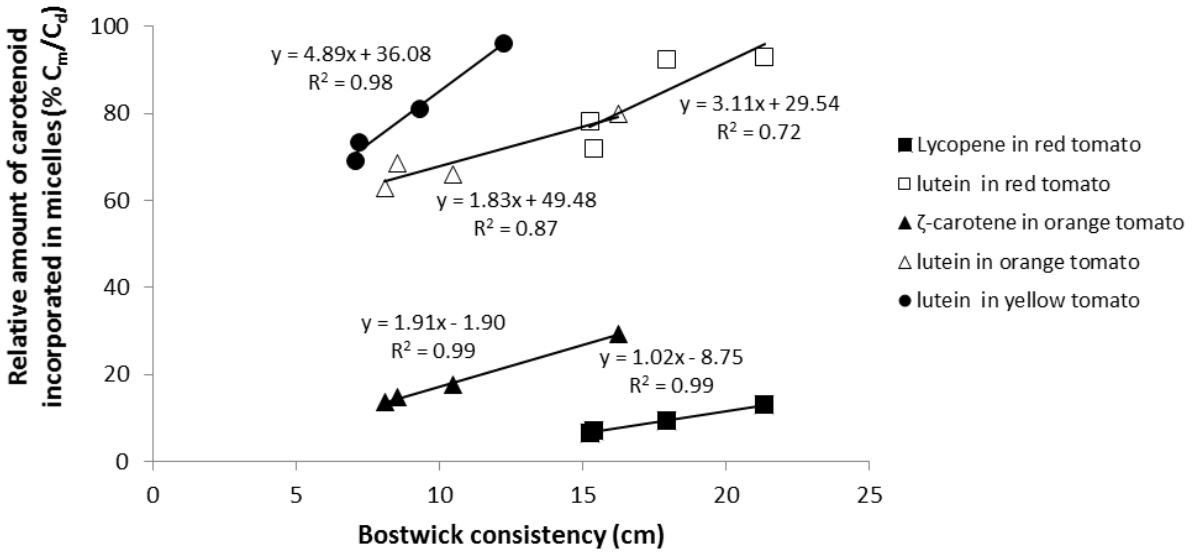


Fig. 3. Light micrographs of chromoplasts of tomato pulps (magnification: 100x). From top to bottom: red, orange and yellow tomato pulp. From left to right: non homogenised and homogenised pulps at 20, 50 and 100 MPa.

1

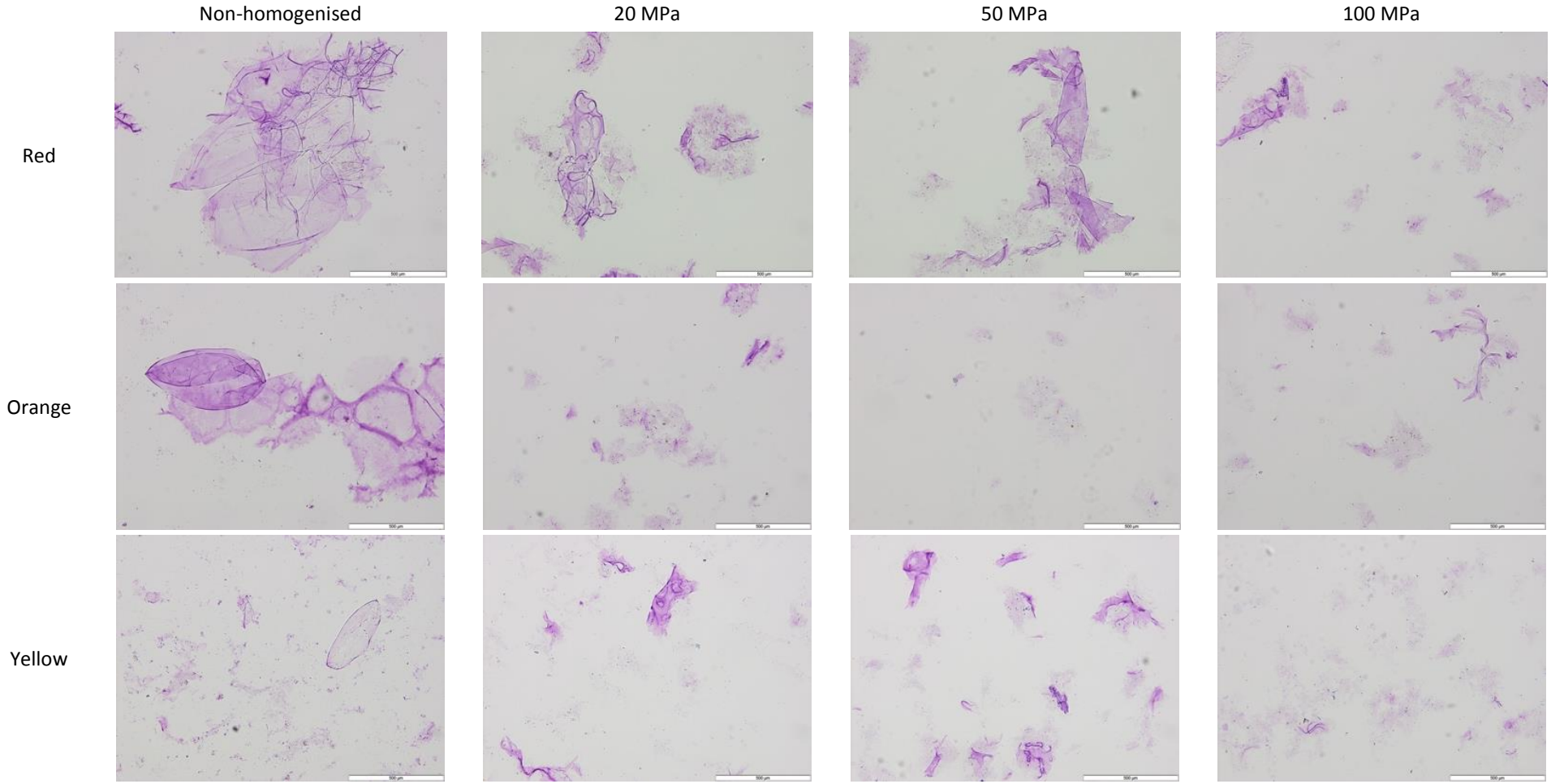


2

3 **Fig. 4** Relative amount of carotenoids incorporated into micelles as a function of the Bostwick

4 consistency index of homogenised tomato pulps. Results of the regression analysis are also shown.

Supplementary material



Light micrographs of tomato pulps stained with toluidine blue (magnification: 10x). From top to bottom: red, orange and yellow tomato pulp. From left to right: non-homogenised and homogenised pulps at 20, 50 and 100 MPa.